# ACTION OF STREPTOCOCCUS FAECALIS CHAIN DISRUPTION SYSTEM AS REVEALED BY SCANNING ELECTRON MICROSCOPY

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*SUMMARY : Short-chain Streptococcus faecalis group (D) zymogenes has been described to elaborate a lytic factor in fluid media which disrupts longer chains. The chain disrupting system was fractionated on Sephadex G-75 and its action was studied by scanning electron microscopy; indicating random sites of its attack on cocci of homologous species namely NCTC 2400.*

*Key Word : Streptococcus faecalis.*

## INTRODUCTION

Streptococcus faecalis characteristically grows in short aggregates of chemically unrelated compounds (1) and also of enzyme defective mutants isolated after UV irradiation (2). The phenomenon of long chain formation through inhibition and/or inactivation of the enzyme system had been reported under the influence of chemically unrelated compounds (4) and a physical agent UV (2).

Shaikh (5) described partial purification and a probable mechanism of chain disruption by this enzyme system through phase contrast microscopy. Present study elucidates further on the details of the action of chain disrupting system revealed under scanning electron microscopy.

## MATERIALS AND METHODS

Experimental chain disruption system was used throughout as described by Shaikh (5). Samples were withdrawn after one hour with the help of 10 micro-liter micropipettes. The droplets were placed on the previously cleaned brass stubs of 1 cm diameter previously coated with dotite electroconductives, type D-550, Fugicura Kasae Co. Ltd. Japan, and air dried. Thereafter the stubs were coated with gold on JEOL FINE COAT, ION SPUTTER JFC 110 metal coating system. Six minutes time was allowed to coat the stubs with an angle of 100 degrees. The stubs were then taken to JEOL-JSM T 200 scanning electron microscope.

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## RESULTS AND DISCUSSION

Figure 1 demonstrates preliminary observation as revealed under light microscopy. A is untreated control whereas B is the appearance after one hour contact with partially purified chain disruption system, demonstrating

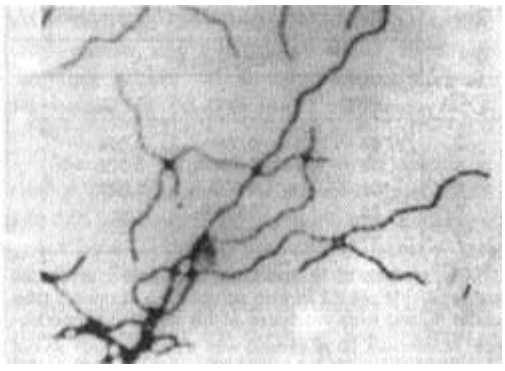


Plate 1: Photomicrographs of chain disruption x 1200
A. Untreated controls.

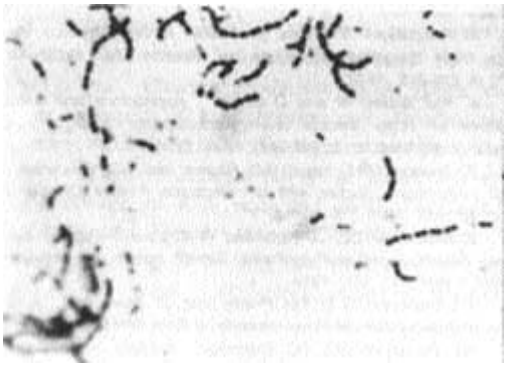
extent of chain disruption by presence of lysed/lysing Gram negative cocci. Based on this observation the chain disruption/lysis phenomenon was studied on scanning electron microscope.

Figures 2 and 3 indicate the untreated controls and the lytic action of the partially purified enzyme system under scanning electron microscope.

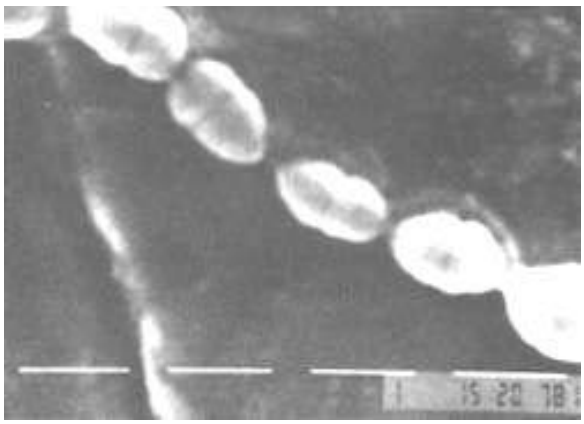
Figure 2a chain of NCTC 2400 observed in buffer control x 10000 : 2B x 20000 : 2C x 50000.

Figure 3 Chain disruption/lysis of NCTC 2400 after one hour exposure with partly purified systems : (A) x 15000, (B) x 20000, (C) x 35000.

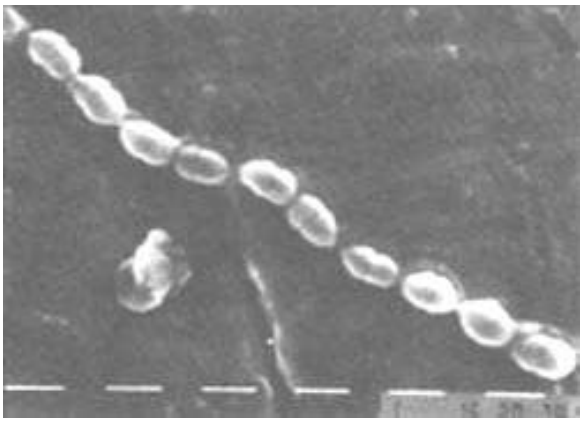
The figures confirm the phenomenon of chain disruption being Lytic. Controls Figure 2 do not show any Lytic activity in form of Gram negative lysing cocci, in light microscopy or dissolution of cells under high magnification scanning electron microscopy. Figure 3 indicates chain disrupting/lytic activity after exposure of chains for one hour with lytic factor. The coccus at lower magnification Figure 3a indicates complete dissolution of a coccus in the meddle of the chain and also partial lysis of the cocci, adjacent to the lysed cell. As magnification is increased the lysis of the coccus became more defined, and remains of the lysed cell can easily be seen. These lytic sites are random and ultimately lead the cell to completely lyse and thus eventually impair the chain orientation (5). Present scanning electron microscopic studies elucidate in detail on the mechanism of chain disruption and confirm the early findings of Lominski, Cameron and Wyllie (4). Shaikh (5) and Shaikh (6) reported the enzyme system's accumulation in fluid media and gradual increase in the lytic activity as the culture progressed, the maximum being close to



b) Chain disruption lysis after exposure with one hour.



b) Chain x 20.000



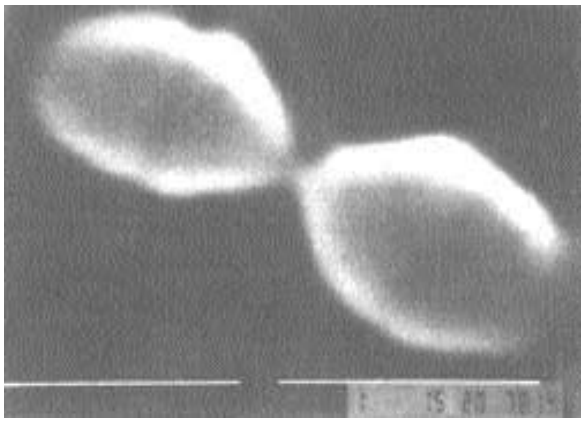
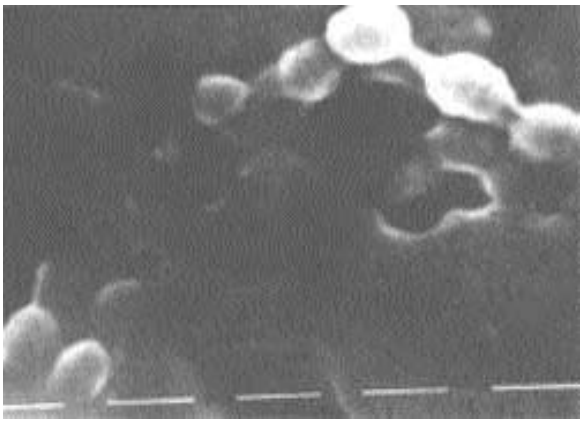


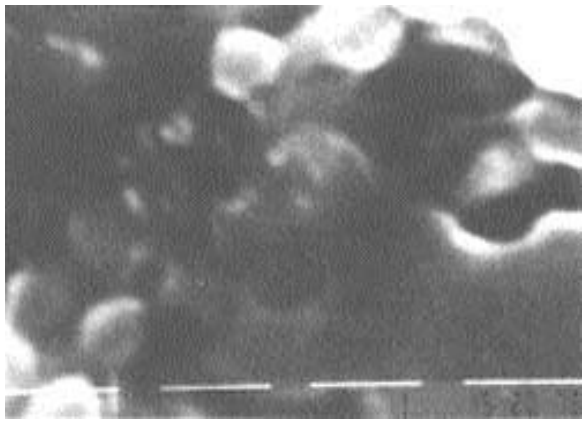
Plate 2: Appearance of NCTC 2400 in buffer controls.

a) Chain x 10.000

c) Chain x 50.000

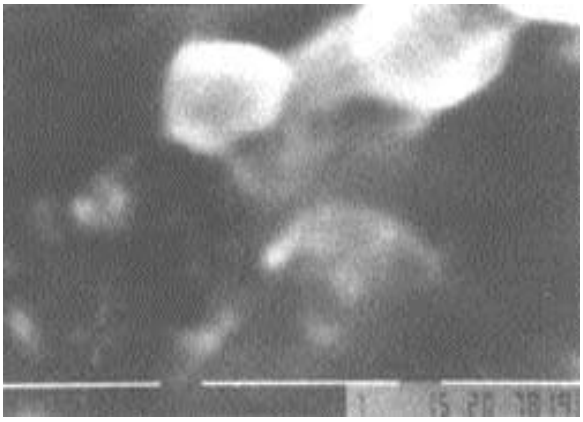


a) x 15.000



b) x 20.000

Plate 3: Chain disruption lysis of cocci NCTC 2400.



c) x 35.000

stationary phase. These findings of chain disruption are in conformity with earlier work of Toennis *et al.* (7), Shockman *et al.* (8-10).

Indeed Sanchez-Pulles has demonstrated the converse phenomenon of induction of chain formation in Pneumococci under the influence of Amidase inhibition (11). The present lytic system leading to chain disruption and lysis does not appear to be the same as reported by Pooley *et al.* (12) as ATCC 9790 had been later recognized as S. faecium.

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